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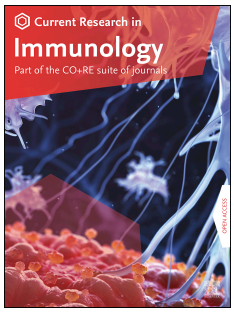

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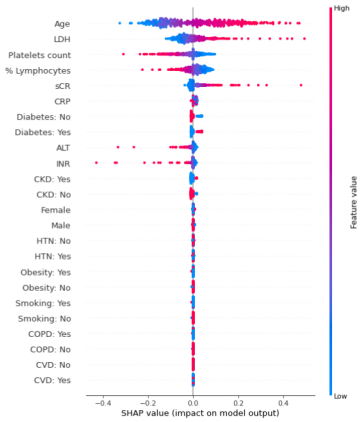

Immune and cellular damage biomarkers to predict COVID-19 mortality in hospitalized patients

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ABSTRACT

Early prediction of COVID-19 in-hospital mortality relies usually on patients' preexisting comorbidities and is rarely reproducible in independent cohorts. We wanted to compare the role of routinely measured biomarkers of immunity, inflammation, and cellular damage with preexisting comorbidities in eight different machine-learning models to predict mortality, and evaluate their performance in an independent population. We recruited and followed-up consecutive adult patients with SARS-Cov-2 infection in two different Italian hospitals. We predicted 60-day mortality in one cohort (development dataset, n=299 patients, of which 80% was allocated to the development dataset and 20% to the training set) and retested the models in the second cohort (external validation dataset, n=402).

Demographic, clinical, and laboratory features at admission, treatments and disease outcomes were significantly different between the two cohorts. Notably, significant differences were observed for %lymphocytes ($p<0.05$), international-normalized-ratio ($p<0.01$), platelets, alanine-aminotransferase, creatinine (all $p<0.001$). The primary outcome (60-day mortality) was 29.10% (n=87) in the development dataset, and 39.55% (n=159) in the external validation dataset. The performance of the 8 tested models on the external validation dataset were similar to that of the holdout test dataset, indicating that the models capture the key predictors of mortality. The *shap* analysis in both datasets showed that age, immune features (%lymphocytes, platelets) and LDH substantially impacted on all models' predictions, while creatinine and CRP varied among the different models. The model with the better performance was model 8 (60-day mortality AUROC 0.83±0.06 in holdout test set, 0.79±0.02 in external validation dataset). The features that had the greatest impact on this model's prediction were age, LDH, platelets, and %lymphocytes, more than comorbidities or inflammation markers, and these findings were highly consistent in both datasets, likely reflecting the virus effect at the very beginning of the disease.

1. INTRODUCTION

The outbreaks of the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first detected in Wuhan, China, in December 2019, evolved into a pandemic in the following weeks, raising concerns all over the world (Huang et al., 2020a). The infection can lead to coronavirus disease 2019 (COVID-19), which is characterized by a high rate of hospitalization, respiratory failure, and ultimately death (W. Guan et al., 2020; Onder et al., 2020; Zhou et al., 2020). To improve the recognition of the patients at higher risk of deterioration and death, efforts were undertaken to early predict the outcomes, ideally at the point of hospital admission.

Numerous articles on large cohorts of hospitalized patients affected by COVID-19 have been published so far (Geleris et al., 2020; Grasselli et al., 2020; W. Guan et al., 2020; Hamer et al., 2020; Huang et al., 2020b; Richardson et al., 2020; D. Wang et al., 2020; Zhou et al., 2020). Coexisting conditions, such as diabetes, hypertension, malignancy, chronic obstructive pulmonary disease (COPD), obesity and older age are risk factors for severe disease and poor outcome in hospitalized patients (Chow et al., 2020; Docherty et al., 2020; Du et al., 2020; Wei-jie Guan et al., 2020; Huang et al., 2020a; Petrilli et al., 2020; Simonnet et al., 2020; Zhang et al., 2020; Zhou et al., 2020). Along with these clinical predictors, several immune and inflammatory markers predicting worst outcomes have been identified. Patients with severe COVID-19 develop life-threatening hyperinflammatory response to the virus, which is characterized by a high circulating levels of C-reactive protein (CRP) and interleukin (IL)-1β, IL-6, IL-18, tumor-necrosis factor, granulocyte-macrophage colony stimulating factor and interferon-γ (Mehta et al., 2020) (Ruan et al., 2020). This response is detrimental and has been shown to anticipate the intubation and mortality. On the other hand, more severe forms of COVID-19 were associated with peripheral lymphocyte subset alteration, and patients with higher lymphocyte counts were less likely to have cytokine storm syndrome and may experience more harm than benefit when receiving corticosteroids (F. Wang et al., 2020) (Lu et al., 2021). Among others, lactic dehydrogenase (LDH), lymphocyte and CRP have been shown to have a role in the stratification of COVID-19 hospitalized patient outcomes (Brinati et al., 2020; Yan et al., 2020).

With the attempt to offer incremental value for patient stratification to these univariable predictors, machine learning (ML) models were used to achieve a more accurate outcome prediction to support decision making when dealing with critically ill COVID-19 patients (Brinati et al., 2020; Yan et al., 2020). However, these ML models showed the challenges of the prediction of outcomes, since in most cases the reported performance was found to be overestimated in the tested population, when the model was validated in an external one (Gupta et al., 2020).

In this study, we aimed to compare the role of routinely measured biomarkers of immunity, inflammation, and organ damage at hospital admission with preexisting comorbidities in eight different machine learning models to predict 60-

day mortality. Importantly, to assess the generalizability our findings, we aimed to evaluate the models' performance in an unrelated, external population from a different hospital.

2. MATERIAL AND METHODS

2.1 Setting and data sources

We conducted an observational retrospective study collecting 2 independent cohorts, one from Poliambulanza Hospital of Brescia, Italy, referred as the "Brescia cohort", and one from Policlinico San Marco, Hospital of Zingonia, Bergamo, Italy, referred as the "Zingonia cohort". Study participants were consecutive adult (≥18 years old) patients with documented COVID 19 infection (i.e., tested by reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay for SARS-CoV-2) at admission in the internal medicine units, from March 1st to April 1th 2020. Follow-up continued until death or May 31st, 2020. The electronic medical records of the patients recruited were accessed by the respective providers and data were manually abstracted, allowing a detailed case ascertainment.

The study is reported in accordance with transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD) guidance for external validation studies (Collins et al., 2015). This study was conducted in compliance with the Good Clinical Practice protocol and the Declaration of Helsinki principles and was approved by the local institutional review board.

2.2 Case ascertainment and variable assessed

Laboratory exams and clinical data were withdrawn and collected at day 1 of patient admission (baseline). Treatment and outcome data were collected during the follow up, from day 1 forward. Severe patients that at admission which were deemed to be hospitalized directly in ICU were not included. Patients with clear evidence of bacterial pneumonia (i.e. clear imaging signs of bacterial pneumonia according to the radiological report) were also excluded.

Patients were treated for COVID-19 according medical judgment, following slightly different protocol in the two hospital. In the Poliambulanza hospital of Brescia, treatment option included hydroxychloroquine (HCQ) 200 mg/day; oral prednisolone or equivalents: 5-25 mg/day. Antiviral therapy (oral Lopinavir/ritonavir, 400 mg/100 mg 2 times/day) were available, and biologic therapy (subcutaneous tocilizumab, 162 mg single shot, eventually repeated after 12 hours if no response were observed). In the Zingonia hospital, antiviral and biologic therapy were not available, while HCQ and Prednisone were variably used. Both structures used antibiotics, in the majority of cases azithromycin 500 mg/day or oral cefixime: 400 mg/day. In general, patients started with azithromycin with or without HCQ, and cefixime was added after 5 days if no improvement was seen, in case of macrolide allergy or in addition to previous treatments in patients with age ≥65 or ≥1 comorbidities. Prednisolone and HCQ were added according to clinical judgment. Low-flow O_2 therapy were prescribed to patients with oxygen saturation <93% at resting in ambient air documented by pulse oximeter (<88% for patient affected by COPD) or heart rate >22 beats per minute. Data on patients' demographic, baseline comorbidities, presenting symptoms, oxygen saturation in ambient air at presentation, historical and current medication list, low-flow O_2 prescription by the general practitioners, inpatient hospitalization, invasive and non-invasive ventilator use data, and death were collected.

2.3 Variables of interest and outcome

Categorical and continuous variables already shown to have a prognostic value for COVID-19 patients were collected. Blood hypertension (HTN), smoking (current or former) ≥10 pack/year, chronic obstructive pulmonary disease (COPD), cardiovascular diseases (coronary artery disease, heart failure, atrial fibrillation), diabetes, and chronic kidney disease (CKD) ≥ grade III (eGFR<60ml/min/1.73 m^2), were identified and recorded as present or absent according to chart review. Age and sex were also included. The most recent patient weight and height, during the 12 months preceding the admission to the hospital were collected, and BMI was calculated; following the World Health Organization definitions (World Health Organization, n.d.) , obesity was defined as having a BMI ≥ 30kg/m^2 (World Health Organization, n.d.). A routine panel of laboratory exams were performed at patients' admission, including complete blood cell count, LDH, CRP, serum creatinine (sCr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and international normalized ratio (INR).

The eight numerical variables included in the "Numerical" models were: age at diagnosis, lymphocytes percentage, platelets count, CRP, LDH, ALT, sCr, INR. In the "Numerical and Categorical" models the following eight categorical variables were added to the previous numerical ones : sex, obesity, diabetes, HTN, COPD, CKD, cardiovascular disease, smoking.

The primary endpoint of this study was 60-day mortality. The time from index date (hospital admission) to death was also collected. Other outcomes collected were the need of O_2 therapy, the need of non-invasive ventilation (NIV), the need of intubation in intensive care unit (ICU) during the observation period.

2.4 Data curation and statistical Analysis

Categorical data were summarized as percentages, significant differences between the 2 independent cohorts or associations of outcomes with clinical features were analyzed using the X^2 test or Fisher exact tests, where appropriate. Continuous variables were presented as mean±standard deviation (SD) or median and interquartile range (IQR), depending on normality demonstrated by Kolmogorov–Smirnov test. Comparisons were performed with Student's t-test for independent samples (2-tailed). Kaplan-Meier survival plots were constructed and the survival curves for groups were compared using a log-rank test. Patients without a primary endpoint event had their data censored on May 31st, 2020.

All the analyses were performed using JMP Pro package (SAS Institute Inc., Cary, North Carolina) and SAS System for Windows, version 9.4 (SAS Institute), and scikit-learn (Pedregosa et al., 2011). A p-value of <0.05 was considered statistically significant for all the analysis.

All data processing was performed using scikit-learn (Pedregosa et al., 2011). In case of missing data, missing values were imputed using the *Iterative Imputer* functions, that models each feature with missing values as a function of other features in a round-robin fashion (Buuren and Oudshoorn, 2011). The Brescia cohort was randomly divided into a training and a test set: 80% of the Brescia cohort served as training set and 20% as test set. All the data from the Zingonia cohort served as the external validation dataset. After the train/test split, we normalized the numerical features of the training data using the *Standard Scaler* function, that standardize each feature by removing the mean and scaling to unit variance; for categorical variables, we performed *one-hot encoding* using the *One Hot Encoder* function. We applied the transformations learned on the training set on the two test sets (Brescia and Zingonia).

2.5 ML Models: development, training, evaluation and interpretability

We evaluated four machine learning classifiers:

- Decision tree (DT) (Safavian and Landgrebe, 1991)
- Random forest (RF) (Ho, 1998)
- Gradient boosting (GBOOST) (Friedman, 2001)
- Support vector machine (SVM) (Williams, 2003)

All classifiers were developed in Python using the scikit-learn library (Pedregosa et al., 2011). We trained each of the four classifiers using only numerical features, or a combination of numerical and categorical features, for a total of 8 models.

Prior its training, each classifier required the definition of a set of parameters that will drive the training process (hyperparameters). To find the best combination of hyperparameters for each model, we performed a grid search analysis using a nested five-fold cross-validation on the training set, using the mean F1-score obtained in the five folds as the metric to select the best performing hyperparameters; we then used the selected parameters to re-train each model from scratch on the whole training set. Given the imbalance between the two classes being predicted, we also tested different combination of *class weights* to help the models focusing on the minority class.

After cross-validation, a total of 8 best-performing models (two for each classifier) were selected and used to perform predictions on both test sets (**Supplementary Figure 1**). We evaluated the models using precision, recall, F1-score, and AUROC. These were defined as follow:

- Precision = True Positive / (True Positive + False Positive)
- Recall = True Positive / (True Positive + False Negative)
- F1 Score = 2 * [(Precision * Recall) / (Precision + Recall)]

We used the python package *shap* (Lundberg et al., 2018) to interpret the output of our models, and have a sense of the features that most influence the models' predictions. Briefly, SHAP (SHapley Additive exPlanations) uses classic Shapley values from game theory and their extension to connect optimal credit allocation with local explanation and assigns each feature an importance value for a particular prediction, allowing interpreting the predictions of complex models (Lanctot et

al., 2017). We used the *shape* package to obtain the summary plots that show which features contributed the most to the model's predictions. We performed this detailed analysis on the model that showed the best performance on the external validation set.

3. RESULTS

3.1 Demographic, clinical, and laboratory features at admission, treatments and outcomes were significantly different in the two datasets

A total of 302 and 411 patients were included from the Brescia and Zingonia cohorts, respectively. We excluded 3 and 9 patients respectively because they did not meet the inclusion criteria (i.e., evidence of bacterial pneumonia). A total of 299 and 402 patients, respectively, were therefore included in the analysis. For the model development, we allocated 80% of the patients (n=239) of the Brescia cohort as training set and 20% of the patients (n=60) as test set. The complete Zingonia cohort (402 patients) was used as the external validation dataset. The study design is summarized in **Supplementary Figure 1**.

Baseline demographic and clinical features are described in **Table 1**. The frequency of obesity, smoking and the CKD ≥III grade were higher in the development dataset (<0.0001). At admission, the proportions of patients with fever>37.5°C and subjective dyspnea at resting were significantly lower in the development dataset, while the PaO_2/FiO_2 ratio were significantly higher in the validation dataset. Significant difference between the two datasets were observed for % of lymphocytes ($p<0.05$), platelets count ($p<0.0001$), alanine aminotransferase ($p<0.0001$), international normalized ratio ($p<0.01$), creatinine ($p<0.001$), but not for white blood cell count, C-reactive protein, lactic dehydrogenase (LDH), aspartate amino transferase ($p>0.05$). Treatment approach was significantly different as well: the frequency of antibiotics, HCQ and Prednisone was significantly lower in the validation cohort, and antiviral and biologic therapy was never used to treat these patients. As a consequence, the proportion of patients requiring NIV and the proportions of deaths were significantly higher in the validation cohort compared to the development one.

3.2 Baseline clinical and laboratory features in survivors versus non-survivors were similarly distributed in the two datasets

The features included in the models are represented by outcomes for each dataset in **Figure 1**. For clinical variables, age of the patients and the proportion of HTN, CVD, diabetes, and CKD were significantly higher in those that died during the 60-day observation period in both the datasets (**Figure 1A** for the development dataset, **Figure 1B** for the

validation dataset). Similarly, lymphocyte percentage, CRP, LDH and sCr levels were higher in those patients that met the primary outcome (**Figure 1C** for the development dataset, **Figure 1D** for the validation dataset). Data imputation was very low (<0.01% in total, single variable ranging from 0 to 0.04%).

3.3 Model training and evaluation in the development dataset and performance in the external validation set

The 8 models were developed and were evaluated using F1-score and AUROC (**Supplementary Table 1**). When predicting the 60-days mortality after hospitalization in the test set, the performance was heterogeneous among the different models (**Supplementary Table 2**). Model 3 (GBOOST numerical) achieved the highest mean F1-score (weighted avg 0.83) followed by Model 7 (SVM, numerical; weighted avg 0.79), Model 8 (SVM numerical and categorical, weighted avg 0.78) and Model 5 (RF Numerical, weighted avg 0.78), Model 4 (GBOOST Numerical and Categorical, weighted avg 0.74), Model 2 (DT Numerical and Categorical, weighted avg 0.73) and Model 6 (RF Numerical and Categorical, weighted avg 0.73). Model 1 performed badly (DT Numerical, weighted avg 0.49).

Compared to the internal test set, the mean F1 scores on the external validation set were lower for all the models (**Table 2**). Model 8 (SVM numerical and categorical) achieved the highest mean F1 score (weighted avg 0.72) followed by Model 6 (RF categorical, weighted avg 0.71) and Model 7 (SVM, numerical; weighted avg 0.70). All the other Models had a mean F1 score between 0.60 and 0.67, except Model 1, which performed worse (DT Numerical, weighted avg 0.49). Overall, although less accurate, the performance of the 8 tested models on the external validation dataset were similar to that of the holdout test dataset, indicating that the models capture the key predictors of patient mortality.

3.4 Immune and laboratory features at hospital admission impacted on mortality prediction more than concomitant clinical comorbidities or hyperinflammation

To make these ML models explainable in terms of the weight of each individual feature tested (i.e. age, sex, patient preexisting comorbidities, immune e laboratory parameters at hospital admission) for patient survival, we performed the *shap* analysis on all the 8 models in both the development test set and the external validation dataset (**Supplementary**

Figure 2 and 3). The *shap* analysis automatically orders the variables used based on the impact of each variable on the model output. In all models and of both the datasets, immune features (%lymphocytes, platelets), cellular damage (LDH in particular) substantially impacted on the models since were constantly among the first positions the ranking. In all the models but one, age impacted significantly (first in the ranking), while the effect of sCr and CRP varied among the different models. Beside age, the weight of the preexisting comorbidities was substantially lower compared to laboratory features.

Given its better performance on the external validation set, we focused our further evaluations on Model 8, a SVM classifier that uses both numerical and categorical variables; the hyperparameters for this model are listed in the **Supplementary Table 3.** This model had an AUROC for 60-day mortality of 0.83±0.06 in the holdout test set, and an AUROC of 0.79±0.02 in the external validation dataset (**Supplementary Figure 3**). When considering the contribution of each of the features in this model, in both the development test set and the external validation dataset (**Figure 2A and 2B,** respectively), age at admission had the greatest impact on the predictions, with older age driving the predictions towards deaths and younger age driving the predictions towards survival. This was followed by LDH (with higher levels driving prediction towards death), platelets count and %lymphocytes (with lower levels driving prediction towards death). The weight of these variables on the model predictions was highly consistent in both the datasets. Serum creatinine had also a significant weight in both dataset (with higher levels driving prediction towards death), while CRP did only the external validation dataset.

4. DISCUSSION

Early prediction of COVID-19 in-hospital mortality relies usually on preexisting comorbidities and is rarely reproducible in independent cohorts of hospitalized patients. Our findings showed that immune and cellular damage markers at hospital admission impacted on mortality prediction substantially more than the presence of concomitant clinical comorbidities or systemic inflammation features (such as high CRP), and these results were reproducible in an independent population with different baseline features and outcomes. Numerous articles on hospitalized patients affected by COVID-19 showed that diabetes, hypertension, malignancy, COPD, obesity and older age are risk factors for severe disease and poor outcome in hospitalized patients (Chow et al., 2020; Docherty et al., 2020; Du et al., 2020; Wei-jie Guan et al., 2020; Huang et al., 2020a; Petrilli et al., 2020; Simonnet et al., 2020; Zhang et al., 2020; Zhou et al., 2020), while the role of immune and

other laboratory parameters in mortality prediction were not reported so often. Patient with severe COVID-19 develop life-threatening hyperinflammatory response to the virus, which is characterized by a high circulating levels of CRP and IL-1β, IL-6, IL-18, tumor-necrosis factor, granulocyte-macrophage colony stimulating factor and interferon-γ. This response is detrimental and has been shown to anticipate the intubation and mortality (Mehta et al., 2020) (Ruan et al., 2020). However, the attempt of blocking hyperinflammation with available agents inhibiting IL-6 (tocilizumab, sarilumab) and IL-1 (anakinra) has led to conflicting and ultimately marginal results in both clinical trials and real word settings (Campochiaro et al., 2020; Cavalli et al., 2020; Della-Torre et al., 2020; Guaraldi et al., 2020; Salvarani et al., 2021; Stone et al., 2017), suggesting that these agents may have a limited role in controlling the disease. On the other hand, more severe forms of COVID-19 were associated with peripheral lymphocyte subset alteration, and patients with higher lymphocyte counts were less likely to have cytokine storm syndrome and may experience more harm than benefit when receiving corticosteroids (F. Wang et al., 2020) (Lu et al., 2021). Consistently, CD8+ T cells tended to be an independent predictor for COVID-19 severity and treatment efficacy (F. Wang et al., 2020). In other studies, markers of cellular damage and in particular LDH has been shown to have a role in the stratification of COVID-19 hospitalized patient outcomes (Brinati et al., 2020; Yan et al., 2020). In our study, beside age, immune and laboratory features at hospital admission impacted on mortality prediction substantially more than the presence of concomitant clinical comorbidities or hyperinflammation. Taken altogether, we can speculate that this probably reflects the effect of the virus at the very beginning of disease onset, while the prediction of the risk may change dynamically during the disease and hospitalization course, i.e. as in ICU cohorts in which comorbidities may impact much more on patient survival or life-threatening hyperinflammatory response to the virus is usually reflected by higher circulating levels of CRP, IL-1β, IL-6, IL-18, and interferon-γ. Of course, other factors may be involved, as for example the genetic background of the patients or the virus genetic variant affecting patients.

In a rapidly evolving field like the COVID-19 research, discoveries accumulate rapidly. The strength of our approach is that it allows to interpret the clinical and laboratory variables imputed to perform a prediction, possibly favoring the selection of biomarker candidates for prospective trials. From this perspective, these models showed their potential as discovery tools rather than clinical tools, and their interpretable features makes them great candidates for this application. One thing to consider is the feasibility of incorporating the recent discoveries in a model like ours, that has built by imputing data from clinical routine. Recently, new potential immunologic biomarkers with prognostic value for COVID-19, such as mucosal-associated invariant T (MAIT) cells (Flament et al., 2021) or circulating NKT cells (Kreutmair et al., 2021), have been discovered. The methodology we used, i.e. the ML modelling, can be easily applied to these variables, contributing to reveal

the immune dysregulation occurring during COVID-19 infection and with potential prediction of the outcome. The limitation of these ML modelling is that large numbers of patients are usually required to avoid overfitting.

The early prediction of the prognosis of COVID-19 patients is of global interest. Much effort has been undertaken to understand which patients are at higher risk of deaths, in order to intensify treatment and care in these individuals. The growing body of literature offers many examples of studies aiming to stratify COVID-19 patients for early mortality prediction, by means of ML algorisms (Brinati et al., 2020; Gupta et al., 2020; Yan et al., 2020) or more conventional regression models (Chow et al., 2020; Docherty et al., 2020; Du et al., 2020; Wei-jie Guan et al., 2020; Huang et al., 2020a; Petrilli et al., 2020; Simonnet et al., 2020; Zhang et al., 2020; Zhou et al., 2020). Since none of the clinical or laboratory variables taken singularly was able to indisputably stratify the outcome of these patients at admission, several ML models were published. ML models have shown a great potential in predicting COVID-19 outcome and perform COVID-19 diagnosis (Chow et al., 2020; Docherty et al., 2020; Du et al., 2020; Geleris et al., 2020; Grasselli et al., 2020; W. Guan et al., 2020; Wei-jie Guan et al., 2020; Hamer et al., 2020; Huang et al., 2020a, 2020b; Petrilli et al., 2020; Richardson et al., 2020; Simonnet et al., 2020; D. Wang et al., 2020; Zhang et al., 2020; Zhou et al., 2020). A common limitation of ML models is that they might overfit to the population used to develop them, resulting in poorer performance when tested in different ones. The issue of overfitting has recently emerged also for COVID-19, since 22 published models, specifically developed for COVID-19 or routinely used in the clinical activity to assess the severity of pneumonia or general status (e.g. CURB65, NEWS2, etc.) performed sub-optimally when validated in an external cohorts (Gupta et al., 2020). It should also be noticed that most of these models were developed in a single center and not tested in an external population during the publication process, and that AUROC was used to assess their net benefit, both potentially leading to imprecision. Our work is unique since we had the opportunity work on 2 independent datasets, one used for development and one for external validation. This conferred robustness to our analysis. We developed and validated 8 models to predict 60-day mortality in two independent cohorts of hospitalized patients with COVID-19. We evaluated our models using the F1 score, a metric that considers both false positives and false negatives into account, and it is more accurate in the case of an uneven class distribution of the outcome, as in our case. Model 8 (SVM Numerical and Categorical) showed the best F-1 score on the external validation dataset, indicating the best performance, which corresponded to an AUROC of 0.79. To ensure comparability with previous ML models (Gupta et al., 2020), we calculated AUROC for Model 8 in the external validation population. The average of AUROCs were 0.60 of all the previous models when assessing mortality, with the highest being 0.76 for the models REMS and Xie (Gupta et al., 2020). Of note, the reason why nobody so far obtained a valid and reproducible prediction might be that the conventional parameters used for the modeling are not sufficient, and maybe

more-disease specific features are needed to predict mortality, and this might be particularly true for patient preexisting comorbidities. Overall, even if the ultimate goal of ML modelling is the development of a risk prediction model at an individual patient level, collectively taken, most of these models failed the predictions in clinical practice. Although ML tools developed to assist in the management of COVID-19 have demonstrated high potential, the great majority of them (if not all) are not routinely used to support clinical decision making. The reasons might be many, i.e. the incapacity of the models to account for the changing nature of the predicted outcomes, or some of the input features do not have the anticipated impact on the predictions because rarer or less discriminating than expected. In this sense, we are aware of these limitations of ML, and to mitigate these potential issues we tested our models in a second, independent cohort of patients. Altogether, we believe that ML models should be considered research tools rather than tools ready to be deployed in clinical practice. The best use of these models is probably to drive research questions, expand our knowledge of the disease, and to identify potential biomarkers by focusing on the variables that have shown to be the most important in the models' predictions, to be tested in prospective studies. It is important to underline that this is possible only thanks to the complementary interpretability tools, that serves as agents that we can use to debug our models.

Finally, ML models tend to suffer whenever there is a change in either the input data or the population (i.e. population specific characteristics, like age and other demographics, comorbidities, etc.), but also changes in clinical practice, for example with the introduction of new drugs or therapeutic schemes. A possible application of our approach is that, given the interpretability of our models, we could test how they "react" to a change in clinical practice (e.g., will the same variables be important for prognosis?). In conclusion, while we wouldn't advise introducing these models in the clinical practice yet, they could be used experimentally to predict how patients respond to new therapies and, in general, to the improvement in the clinical management of these patients.

This study has some strengths and limitations. Compared to other previous paper, our work is characterized by a very low percentage of data imputation, a clear interpretability and an independent external validation dataset which increases the methodological rigor of our study and allows to test the reproducibility of the models. Most if not all the previous cohorts used for modeling were single-center, retrospective cohorts. Second, we used the nested cross validation and used mean F1 score instead of AUROC to select the models, contributing to the methodological rigor our analyses. A weakness of the current study is the observational retrospective design and the extraction of data from non-standardized medical records cannot completely exclude classification error. In addition, even if missing data were minimal (< 5%), multiple imputation was performed. Laboratory data were collected only at baseline, and not longitudinal data were

retrieved, likely reducing the performance of the tested models. However, most prognostic scores are intended to predict outcomes at the point of hospital admission.

In conclusion, beside age, in our ML models immune and laboratory features at hospital admission impacted on mortality prediction substantially more than the presence of concomitant clinical comorbidities or the presence of a systemic inflammatory status, and these findings were highly reproducible in independent populations. We can speculate that this probably reflects the effect of the virus at the very beginning of disease onset, while the prediction of the risk may change dynamically during the disease course. Future clinical and basic science studies are needed to have a better understanding of the immune and cellular perturbations that occurs during COVID-19, which may help to develop reliable and reproducible prognostic models for COVID-19.

FIGURE LEGENDS.

Figure 1. Clinical and laboratory features of the development dataset (A an C, in the blue panels) and of the validation dataset (B and D, in the white panels) by outcomes. * <0.05, ** <0.01, *<0.001.**

Figure 2. The impact of the input features on predictions. The *shap* analysis on the model with the best precision (Model 8), in the development test set (A) and the external validation dataset (B). The model includes both continuous and binary input features. Continuous features vary from low to high values, whereas binary features are either present or absent. Each dot represents the impact of a feature on the mortality prediction for one patient at entrance. The color indicates the level of contribution of each variable (with red indicating a higher impact on the prediction) and the direction the prediction towards death (right) or survival (left).

SUPPLEMENTARY FIGURE LEGENDS.

Supplementary Figure 1. Study overview and design.

Supplementary Figure 2. Model 8 (SVM Numerical and Categorical). The area under the ROC curve (AUROC).

Supplementary Figure 3. The *shap* analysis on all the 8 models in the development test set.

Supplementary Figure 4. The *shap* analysis on all the 8 models in the external validation dataset.

TABLES

Table 1. Baseline demographics, comorbidities, clinical features at presentation, treatments and outcomes of hospitalized patients with COVID-19 in the development dataset and external validation dataset. The variables used as input variables of the models are marked as *. Comparisons were performed with either X^2 test or Fisher exact tests for categorical variables, and Student's t-test for continuous variables.

Characteristics	Development dataset	External validation dataset	p value
N.	*299*	*402*	
Demographics			
Age at diagnosis, * mean (±SD)	68.79 (11.65)	70.21 (13.17)	0.1384
Male sex,* % (number)	69.57% (208)	67.41% (271)	0.5446
Obesity,* BMI≥30kg/m^2, % (number)	19.40% (58)	5.22% (21)	<.0001
Ethnicity, white,% (number)	99.33% (297)	100% (402)	0.1816
Smoking,* (≥10 pack/year), current or former, % (number)	15.39% (46)	3.48% (14)	<.0001
Comorbidities			
Diabetes,* % (number)	19.39% (58)	19.90% (80)	0.8686
HTN,* % (number)	53.51% (160)	46.77% (188)	0.0773
Cardiovascular Diseases,* % (number)	28.09% (84)	24.13% (97)	0.2356
CKD ≥ stage III,* % (number)	36.12% (108)	7.46% (30)	<.0001
COPD,* % (number)	6.35% (19)	9.70 (39)	0.1116

Cancer (active or < 5 years), % (number)	5.69% (17)	6.22% (25)	0.7686
Previous stroke,% (number)	3.34% (10)	0.50% (2)	0.0041
Clinical presentation			
Fever, temperature>37.5°C,% (number)	85.62% (256)	98.01% (394)	<.0001
Dry cough,% (number)	51.51% (154)	NA	-
Dyspnea at resting, % (number)	50.17% (150)	96.52% (388)	<.0001
Myalgias, % (number)	NA	95.27% (383)	-
Gastrointestinal symptoms, % (number)	6.02% (18)	4.48% (18)	0.3602
Syncope/Presyncope, % (number)	4.01% (12)	NA	-
Altered mental status, % (number)	2.68% (8)	NA	-
Evidence of pneumonia at thoracic imaging,[&] % (number)	96.66% (289)	95.52% (384)	0.4486
PaO_2/FiO_2 Ratio	248.9 (73.6)	355.6 (116.1)	<.0001
Laboratory Characteristics			
WBC, mean (±SD)	7.89 (4.35)	8.13 (4.32)	0.4637
Lymphocytes,* % of WBC, mean (±SD)	14.75 (9.45)	13.28 (7.73)	0.0235
PLT,* mean (±SD)	187.000 (82.000)	225.000 (98.000)	<.0001
CRP,* mean (±SD)	126.3 (88.58)	122.8 (95.7)	0.6260
LDH,* median [25-75%IQR]	395 [305.75-530]	405 [304-524]	0.9897
AST, median [25-75%IQR]	53[38-75]	50 [36-74.25]	0.1225
ALT,* median [25-75%IQR]	32 [20-57]	41 [27.75-62]	<.0001
INR,* median [25-75%IQR]	1.01 [0.96-1.12]	1.04 [0.99-1.12]	0.0018
sCr,* (mg/dL), mean (±SD)	1.26 (0.94)	1.53 (1.13)	0.0011
Treatments			

Antibiotics, % (number)	83.28 % (249)	28.61% (115)	<.0001
HCQ, % (number)	22.75 % (68)	5.72% (23)	<.0001
Lopinavir/ritonavir, % (number)	21.07 % (63)	0% (0)	<.0001
Prednisone, % (number)	34.45% (103)	0.75% (3)	<.0001
Tocilizumab, % (number)	4.01% (12)	0% (0)	<.0001
Outcomes			
O2 therapy,* % (number)	48.16% (144)	35.57% (143)	0.008
NIV,** % (number)	13.04% (39)	19.65% (79)	0.0207
ICU with intubation,*** % (number)	10.03% (30)	10.70% (43)	0.7762
Death, % (number)	29.10% (87)	39.55% (159)	0.0041

Abbreviations: HTN: Blood hypertension , BMI: body mass index; Cardiovascular Disease: chronic heart failure, myocardial infarction, atrial fibrillation; CKD: chronic kidney disease, stage III correspond to estimated glomerular filtration rate < 60 mL/min; COPD: Chronic obstructive pulmonary disease; WBC: White blood cells, PLT: platlets, CRP: C-reactive protein, LDH: lactic dehydrogenase, AST: aspartate aminotransferase; ALT: alanine aminotransferase; INR: international normalized ratio; sCr: serum Creatinine;; Antibiotics: oral Cefixime: 400 mg/day for ≥ 5 days; oral Azithromycin 500 mg/day for ≥ 5 days; oral Claritromicin 250 mg x 2/day for ≥ 5 days endovenoous Ceftriaxon 2 g/day for ≥ 5 days; endovenous piperacillina/tazobactam 4.5 mg x 3 or 4/day for ≥ 5 da;oral or endovenous Levofloxacin 500 mg/day for ≥ 5 days. HCQ: hydroxychloroquine, 200 mg 12 hours apart for the first 2 doses, then 200 mg/day for ≥ 5 days; Oral Prednisolone or equivalents: range 5-25 mg/day for ≥ 5 days. NIV: Non-invasive ventilation; ICU: intesive care unit. SD=standard deviation.

[&] Thoracic X-ray as a screening test, followed by CT-scan in doubtful cases

**O2 therapy:* administered when saturation were ≤92% at resting in ambient air; *required nasal canula or Venturi mask; NIV: required non-inviasive ventilation;*

***NIV: patients non-responsive to high-flow O2-therapy, requiring*

****ICU with intubation: required intensive care unit hospitalization with intubation.*

Table 2. Mean F1-score and AUROC obtained in the cross-validation on the training set (N = 239)		
	F1-score (mean ± SD)	AUROC (mean ± SD)
Model 1: Decision Tree Numerical	0.60 ± 0.06	0.74 ± 0.11
Model 2: Decision Tree Numerical and Categorical	0.68 ± 0.07	0.83 ± 0.07
Model 3: GBOOST Numerical	0.66 ± 0.06	0.84 ± 0.05
Model 4: GBOOST Numerical and Categorical	0.69 ± 0.04	0.88 ± 0.04
Model 5: Random Forest Numerical	0.69 ± 0.15	0.86 ± 0.07
Model 6: Random Forest Numerical and Categorical	0.69 ± 0.05	0.87 ± 0.04
Model 7: SVM Numerical	0.72 ± 0.05	0.87 ± 0.04
Model 8: SVM Numerical and Categorical	0.68 ± 0.03	0.87 ± 0.03

SUPPLEMENTARY TABLES

Supplementary Table 1. Performance of the 8 models selected after cross-validation: development cohort test set (N = 60)				
Model 1: Decision Tree Numerical				
	Precision	Recall	F1-score	Support
Survival	0.83	0.35	0.49	43
Death	0.33	0.82	0.47	17
Accuracy			0.48	60
Macro avg	0.58	0.59	0.48	60
Weighted avg	0.69	0.48	**0.49**	60
Model 2: Decision Tree Numerical and Categorical				
	Precision	Recall	F1-score	Support
Survival	0.69	0.63	0.76	43
Death	0.50	0.94	0.65	17
Accuracy			0.72	60
Macro avg	0.73	0.78	0.71	60
Weighted avg	0.83	0.72	**0.73**	60
Model 3: GBOOST Numerical				

	Precision	Recall	F1-score	Support
Survival	0.87	0.91	0.89	43
Death	0.73	0.65	0.69	17
Accuracy			0.83	60
Macro avg	0.80	0.78	0.79	60
Weighted avg	0.83	0.83	**0.83**	60

Model 4: GBOOST Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.83	0.79	0.81	43
Death	0.53	0.59	0.56	17
Accuracy			0.73	60
Macro avg	0.68	0.69	0.68	60
Weighted avg	0.74	0.73	**0.74**	60

Model 5: Random Forest Numerical

	Precision	Recall	F1-score	Support
Survival	0.84	0.86	0.85	43
Death	0.62	0.59	0.61	17
Accuracy			0.78	60
Macro avg	0.73	0.72	0.73	60
Weighted avg	0.78	0.78	**0.78**	60

Model 6: Random Forest Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.93	0.65	0.77	43
Death	0.50	0.88	0.64	17
Accuracy			0.72	60
Macro avg	0.72	0.77	0.70	60
Weighted avg	0.81	0.72	**0.73**	60

Model 7: SVM Numerical

	Precision	Recall	F1-score	Support
Survival	0.89	0.79	0.84	43
Death	0.59	0.76	0.67	17
Accuracy			0.78	60
Macro avg	0.74	0.78	0.75	60
Weighted avg	0.81	0.78	**0.79**	60

Model 8: SVM Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.94	0.72	0.82	43
Death	0.56	0.88	0.68	17
Accuracy			0.77	60
Macro avg	0.75	0.80	0.75	60
Weighted avg	0.83	0.77	**0.78**	60

Supplementary Table 2. Performance of the 8 models selected after cross-validation: external validation dataset (N = 402)

Model 1: Decision Tree Numerical

	Precision	Recall	F1-score	Support
Survival	0.70	0.71	0.71	243
Death	0.55	0.53	0.54	159
Accuracy			0.64	402
Macro avg	0.58	0.59	0.48	402
Weighted avg	0.69	0.48	**0.49**	402

Model 2: Decision Tree Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.85	0.56	0.67	243
Death	0.56	0.65	0.57	159
Accuracy			0.57	402
Macro avg	0.70	0.70	0.67	402
Weighted avg	0.73	0.67	**0.67**	402

Model 3: GBOOST Numerical

	Precision	Recall	F1-score	Support
Survival	0.66	0.95	0.78	243
Death	0.76	0.26	0.39	159
Accuracy			0.68	402
Macro avg	0.71	0.61	0.59	402
Weighted avg	0.70	0.78	**0.63**	402

Model 4: GBOOST Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.65	0.93	0.76	243
Death	0.67	0.23	0.35	159
Accuracy			0.65	402
Macro avg	0.66	0.58	0.55	402
Weighted avg	0.66	0.65	**0.60**	402

Model 5: Random Forest Numerical

	Precision	Recall	F1-score	Support
Survival	0.69	0.86	0.877	243
Death	0.66	0.41	0.51	159
Accuracy			0.68	402
Macro avg	0.68	0.64	0.64	402
Weighted avg	0.68	0.68	**0.66**	402

Model 6: Random Forest Numerical and Categorical

	Precision	Recall	F1-score	Support
Survival	0.74	0.80	0.77	243
Death	0.66	0.58	0.62	159

Accuracy			0.71	402
Macro avg	0.70	0.69	0.69	402
Weighted avg	0.71	0.71	**0.71**	402
Model 7: SVM Numerical				
	Precision	Recall	F1-score	Support
Survival	0.73	0.81	0.77	243
Death	0.65	0.55	0.60	159
Accuracy			0.71	402
Macro avg	0.69	0.68	0.68	402
Weighted avg	0.70	0.71	**0.70**	402
Model 8: SVM Numerical and Categorical				
	Precision	Recall	F1-score	Support
Survival	0.81	0.70	0.75	243
Death	0.62	0.74	0.68	159
Accuracy			0,72	402
Macro avg	0.71	0.72	0.71	402
Weighted avg	0.73	0.72	**0.72**	402

Supplementary Table 3. Optimal hyperparameters for the SVM classifier (Model 8)

Hyperparameters	**value**
C	10
class_weight	{1:4}
gamma	auto
kernel	linear

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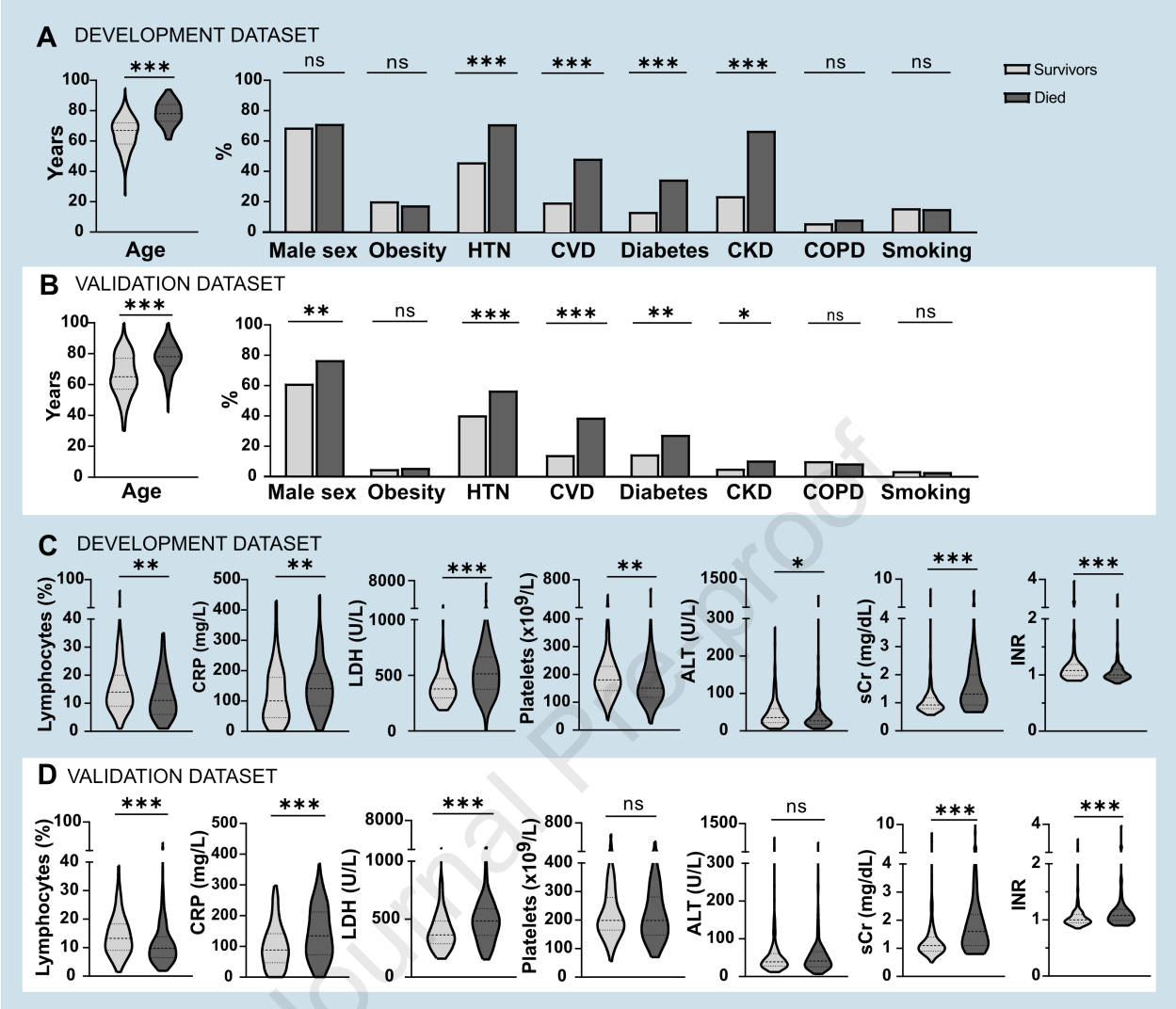

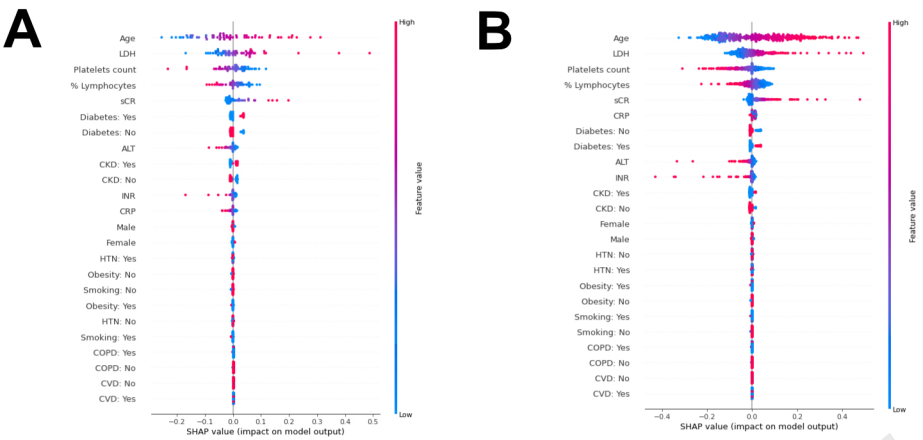

HIGHLIGHTS

- Models for early prediction of COVID-19 mortality relies usually on the presence of preexisting comorbidities, and are rarely reproducible
- In two independent hospital populations, we showed that routinely measured biomarkers relative to the immune response and cellular damage better contribute to the prediction of the prognosis of patients with COVID-19, rather than the preexisting comorbidities
- The features that had the greatest impact on the model's prediction were age, LDH, platelets count, and % of lymphocytes, while creatinine, C-reactive protein and liver biomarkers contributed less constantly to mortality prediction

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: